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BIOLOGY

Altitudinal, phenological, and organ-related Variation in the essential oil profile of *Lavandula stoechas* L. naturally distributed in the Eastern Mediterranean Region

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Variação altitudinal, fenológica e relacionada aos órgãos no perfil do óleo essencial de *Lavandula stoechas* L. naturalmente distribuída na região do Mediterrâneo Oriental

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Altitudinal, phenological, and organ-related Variation in the essential oil profile of *Lavandula stoechas* L. naturally distributed in the Eastern Mediterranean Region

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ABSTRACT: *Lavandula stoechas* subsp. *stoechas* L. is a widely distributed Mediterranean aromatic species, but the factors underlying variation in its essential oil yield and composition remain insufficiently understood. This study evaluated the effects of altitude, phenological period, and plant part on essential oil yield and chemical composition of *L. stoechas* populations in the Eastern Mediterranean Region of Türkiye. Plant materials were collected from four altitudes (150, 300, 450, and 600 m) at two phenological periods (before flowering and full flowering) and separated into leaves and flowers. Essential oil composition was determined by GC–MS, while three-way ANOVA was used to evaluate variation in essential oil yield and principal component analysis (PCA) to assess chemical differentiation among samples. Essential oil yield was significantly affected by altitude, phenological period, and plant part, as well as by interactions involving altitude $P < 0.001$, whereas the period × plant part interaction was not significant. Leaf oil yields ranged from 1.20 to 3.00%, compared with 0.60–1.43% for flowers. The highest yield was recorded in leaves collected at full flowering at 300 m (3.00%). Fenchone was the predominant compound (35.49–64.05%), followed by camphor (3.78–38.17%) and 1,8-cineole (2.33–22.23%). Identified compounds accounted for 80.58–93.18% of the total oil composition. PCA revealed distinct chemical variation among samples, indicating contributions of altitude, phenological period, and plant part to chemotypic differentiation. These findings highlighted the importance of these factors in determining essential oil yield and composition and provide useful information for harvesting and chemotype selection of *L. stoechas*.
Key words: essential oil variability, chemotype, phenological stage, ecological gradient, and GC-MS.

Variação altitudinal, fenológica e relacionada aos órgãos no perfil do óleo essencial de *Lavandula stoechas* L. naturalmente distribuída na região do Mediterrâneo Oriental

RESUMO: *Lavandula stoechas* subsp *stoechas* L. é uma espécie aromática amplamente distribuída na região do Mediterrâneo, no entanto, os fatores que determinam a variação no rendimento e na composição do óleo essencial ainda são pouco compreendidos. Neste sentido, este estudo avaliou os efeitos da altitude, do período fenológico e do órgão vegetal sobre o rendimento e a composição química do óleo essencial de populações de *L. stoechas,* na Região do Mediterrâneo Oriental da Turquia. Para realizar a avaliação, o material vegetal foi coletado em quatro altitudes (150, 300, 450 e 600 m), em dois períodos fenológicos (pré-floração e plena floração), e separado em folhas e flores. A composição do óleo essencial foi determinada por CG–EM, enquanto uma ANOVA trifatorial foi utilizada para avaliar a variação no rendimento do óleo essencial. Além disso, uma análise de componentes principais (ACP) foi realizada para avaliar a diferenciação química entre as amostras. Os resultados revelaram que o rendimento do óleo essencial foi significativamente afetado pela altitude, período fenológico e órgão vegetal, bem como pelas interações envolvendo a altitude ($P < 0,001$), enquanto a interação período × órgão vegetal não foi significativa. Ademais, os rendimentos nas folhas variaram de 1,20 a 3,00%, enquanto nas flores variaram de 0,60 a 1,43%. O maior rendimento foi registrado nas folhas coletadas em plena floração a 300 m (3,00%). A fenchona foi o composto predominante (35,49–64,05%), seguida pela cânfora (3,78–38,17%) e pelo 1,8-cineol (2,33–22,23%). Os compostos identificados representaram 80,58–93,18% da composição total do óleo. A ACP revelou padrões distintos de variação química entre as amostras, indicando a contribuição da altitude, do período fenológico e do órgão vegetal para a diferenciação quimiotípica. Portanto, o estudodestaca a importância dos fatores ambientais e do desenvolvimento na determinação do rendimento e da composição do óleo essencial , além de fornecer informações úteis para a definição do período de colheita e a seleção de quimiotipos de *L. stoechas*.
Palavras-chave: fenchona, cânfora, *Lavandula*, Mediterrâneo Oriental.

INTRODUCTION

Plant secondary metabolites are crucial for coping with abiotic and biotic stresses and constitute key components of plant defense and adaptation mechanisms (KNUDSEN et al., 2006; BAKKALI et al., 2008; DEMASI et al., 2018; DUDAREVA et al., 2013; RAUT & KARUPPAYIL, 2014; NOGUÉS et al., 2015; CASER et al., 2016). Their composition varies with environmental factors such as climate, soil properties, rainfall, and altitude, as well as developmental stage and genetic background (JAN et al., 2021; IZMIRLI & YILDIRIM, 2023; BAHADIRLI et al., 2024), which can influence

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specific biosynthetic pathways (ZHAN et al., 2022). For example, altitudinal gradients can alter temperature and UV-B radiation, potentially affecting enzymatic activities involved in terpene biosynthesis, while developmental changes may modify metabolic allocation between vegetative and reproductive tissues (DOBHAL et al., 2024). Plant volatile organic compounds (VOCs) mediate ecological interactions such as pollinator attraction, herbivore deterrence, and inter-organism communication (BOUWMEESTER et al., 2019). Essential oils, as a major group of plant secondary metabolites; therefore, contributed to plant–environment interactions and associated ecological and physiological functions (PRUSINOWSKA & SMIGIELSKI, 2014).

The Lamiaceae family is one of the major plant families rich in essential oils, and the genus *Lavandula* represents a globally important group of aromatic plants. The genus comprises approximately 39 species distributed from the Mediterranean region to the Arabian Peninsula, Southwest Asia, and India (MSAADA et al., 2012; DEMASI et al., 2018; KEREM & ÖZBEK, 2024). In Türkiye, *Lavandula* has considerable medicinal and economic importance and is naturally represented by two taxa*:* *L. stoechas* subsp. *stoechas* L. and *L. pedunculata* subsp. *cariensis* Mill. (UPSON & ANDREWS, 2004; ŞAHİNLER et al., 2022). Among these native taxa, *L. stoechas*, commonly known as “Karabaş lavanta” in Turkish and Spanish lavender, butterfly lavender, or topped lavender in English, is widely used as an ethnomedicinal resource (BAYTOP, 1994).

The genus comprises evergreen aromatic shrubs with corolla colors ranging from pink and lilac to dark purple (DAVIS, 1978). Cultivated *Lavandula* species have considerable global economic importance, including lavender (*L. angustifolia* Mill., syn. *L. officinalis* Chaix, *L. vera* DC.), spike lavender (*L. latifolia* Mill., syn. *L. spica* DC.), and lavandin (*L.* × *intermedia* Emeric ex Loisel., syn. *L. hybrida* Rev.) (SALEHI et al., 2018). France, Bulgaria, and China are major suppliers of lavender essential oil, with France and Bulgaria accounting for a substantial proportion of global production (KOZUHAROVA et al., 2024). Türkiye has also increased its lavender and lavandin production; although, further development is needed. Total *Lavandula* spp. production is approximately 10,000 tons, while essential oil imports are about nine tons (TUĞLU & BAYDAR, 2026). Approximately 70 tons are also collected from nature as non-wood forest products.

L. stoechas has traditionally been used for the treatment of rheumatic disorders, inflammation, pain, wounds, respiratory ailments, eczema, gastrointestinal inflammation, and neurological disorders, and has also been used as an antiseptic, expectorant, and cardiotonic agent (BAYTOP, 1999; EL-HILALY et al., 2003; EZ ZOUBI et al., 2020). During the Ottoman Empire, *L. stoechas* was considered an important medicinal plant, and historical records indicate its use in the treatment of cholera (BAYTOP, 1999). Although, its essential oils are generally not preferred in perfumery because of their strong odor and high camphor content, both essential oils and solid extracts have been reported to exhibit antioxidant, antispasmodic, antibacterial, antifungal, and insecticidal activities (BENABDELKADER et al., 2011; EZ ZOUBI et al., 2017). Studies from Italy, Portugal, Spain, Morocco, Tunisia, and Türkiye have identified fenchone and camphor as predominant constituents of *L. stoechas* essential oil, while linalyl acetate, α-terpinene, and 1,8-cineole have also been reported as abundant compounds (ZRIRA et al., 2003; ANGIONI et al., 2006; KIRMIZIBEKMEZ et al., 2009; BARHOUCHI et al., 2019).

To optimize the commercial and therapeutic utilization of *L. stoechas*, it is critical to understand how essential oil yield and chemical plasticity respond to environmental gradients and developmental stages (KOCABAŞ OĞUZ & KAPLAN, 2022). The effects of environmental factors and developmental stages on lavender have been investigated by HASSIOTIS et al. (2014), while the effects of latitude and altitude have been examined by DEMASI et al. (2018) and BELLA et al. (2015). However, these studies have largely considered environmental and developmental factors separately and do not fully capture their potential interactions with organ-specific variation. To date, no comprehensive study in Türkiye has systematically evaluated the combined effects of altitudinal gradients, phenological stages, and plant organs on the essential oil profile of wild *L. stoechas* subsp. *stoechas*. This represents an important research gap, particularly in the Eastern Mediterranean, where pronounced environmental heterogeneity may promote substantial chemical variation in aromatic plants (LA BELLA et al., 2015; CHOGRANI et al., 2021; FABER et al., 2026).

This study evaluated variations in essential oil yield and chemical composition of *L. stoechas* subsp. *stoechas*, a species naturally distributed in the flora of Hatay province in the Eastern Mediterranean region, across different altitudinal gradients, phenological stages, and plant parts, thereby providing insights into its physiological adaptation strategies and supporting the identification of conditions associated with specific chemotypes.

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MATERIALS AND METHODS

Plant materials

Lavandula stoechas subsp. *stoechas* samples were collected in 2020 from natural populations at four altitudes in Hatay Province, Eastern Mediterranean Türkiye: Yenicağ (150 m), Alahan (300 m), Nergizlik (450 m), and Karlısu (600 m a.s.l.). Sampling was conducted at two phenological stages, pre-anthesis and full anthesis. Pre-anthesis was defined as fully developed floral spikes with closed calyces and unopened corollas, whereas full anthesis was defined as > 75% of flowers per spike having open calyces and fully expanded corollas.

At each altitude, aerial parts were randomly collected from at least 20 healthy plants separated by ≥ 5 m. Plant identification followed the Flora of Turkey, and a voucher specimen (KN-225) was deposited in the Herbarium of Hatay Mustafa Kemal University. The sampling sites have a Mediterranean climate (Csa), characterized by hot, dry summers and mild, rainy winters, with populations occurring mainly on calcareous, well-drained clay-loam soils (ŞAHİNLER et al., 2022). Samples were shade-dried at 22 ± 2 °C for 14 days and were constantly weighted, separated into leaves and flowers, and stored in paper bags under dry, dark conditions until analysis.

Essential oil extraction

Essential oils were extracted from 100 g of dried herb per sample (three replicates) by hydro distillation for 3 h using a glass Clevenger-type apparatus, following European Pharmacopoeia standards. Essential oil yield was calculated as the volume of oil (mL) obtained per 100 g of dry herb and expressed as a percentage on a dry-weight basis. The oils were dried over anhydrous sodium sulfate (Na_2SO_4), filtered, and stored in airtight amber glass vials at 4 °C until analysis. Results are presented as mean ± standard deviation.

Gas chromatography mass spectrometry

Essential oil components were analyzed using a Thermo Scientific ISQ Single Quadrupole GC–MS system (BAHADIRLI et al., 2024). A 1 µL aliquot of diluted essential oil (5 µL oil in 2 mL cyclohexane) was injected in split mode (1:50). Separation was performed on a TG-Wax capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness) using helium as the carrier gas at 1.0 mL min^{-1}. The oven was initially held at 50 °C and then increased to 220 °C at 3 °C min^{-1}. Mass spectra were acquired in electron ionization mode (70 eV) over m/z 1.2–1200 in full-scan mode. The transfer line and ion source temperatures were 250 and 220 °C, respectively. Compounds were identified by comparison with reference spectra in the Wiley7Nist05, Flavor2, and W9N11 libraries. Essential oil composition was expressed as mean relative peak area percentages.

Statistical analysis

Essential oil yield was analyzed as a three-factorial experiment in a completely randomized design with three replications. The effects of altitude, phenological stage, plant part, and their interactions were evaluated by three-way ANOVA ($P < 0.05$), followed by Tukey's HSD test for mean separation. Analyses were performed in R v4.4.1 using *aov()*, with post-hoc comparisons conducted using *HSD.test()* from the *agricolae* package. Data visualization was performed using *ggplot2*.

Principal Component Analysis (PCA) was performed on standardized relative concentrations (%) of major essential oil constituents using *FactoMineR*. Altitude, phenological stage, plant part, and sample identifiers were included as supplementary variables. The first two principal components were visualized in biplots with 95% confidence ellipses using *factoextra*.

RESULTS AND DISCUSSION

Essential oil content

Statistical analysis showed that altitude, plant part, and phenological stage significantly affected the essential oil (EO) yield of *L. stoechas* subsp. *stoechas* ($P < 0.001$; Table 1). All two-way and three-way interactions were also significant, except for the phenological stage × plant part interaction. These interactions indicate that EO accumulation is influenced by the combined effects of environmental and developmental factors, with the response of EO production to altitude depending on both plant part and phenological stage.

The spatial and temporal variation in EO yield across the studied gradients (Table 2) revealed a non-linear relationship with altitude. The intermediate-altitude populations, Alahan (300 m) and Nergizlik (450 m), exhibited the highest EO accumulation, particularly in leaves at full flowering (FF), reaching 3.00% and 2.00%, respectively, whereas lower yields were recorded at the lowest (Yenicag, 150 m) and highest (Karlisu, 600 m) elevations. Thus, EO production was favored at intermediate altitudes rather than increasing or decreasing linearly with elevation.

The observed distribution is consistent with the stress–secondary metabolism relationship

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Table 1 - Variance sources and statistical analysis results of essential oil content.

Variance sources	Df	Sum Sq	Mean Sq	F value	Significance
Altitude	3	0.872	0.291	22.495	***
Period	1	3.853	3.853	298.323	***
Part	1	8.168	8.168	632.323	***
Altitude × Period	3	1.055	0.352	27.226	***
Altitude × Part	3	1.584	0.528	40.882	***
Period × Part	1	0.007	0.007	0.581	ns
Altitude × Period × Part	3	0.534	0.178	13.785	***
Error	32	0.413	0.013		

***P < 0.001; ns = not significant (P ≥ 0.05).

described for Lamiaceae, whereby moderate environmental stress may stimulate secondary metabolite production, whereas excessive stress can limit metabolic activity and metabolite accumulation (MANSINHOS et al., 2024). Although, environmental variables were not directly measured in the present study, differences among altitudes may reflect changes in microclimatic conditions that influence carbon allocation and terpene biosynthesis. Under intermediate environmental conditions, plants may maintain sufficient physiological performance while simultaneously allocating more resources to secondary metabolism, resulting in greater EO accumulation.

Across all altitudinal gradients and developmental stages, leaf EO content (1.20–3.00%) was consistently higher than floral EO content (0.60–1.43%). This tissue-specific pattern may be associated with differences in the abundance and activity of glandular trichomes and the distinct physiological roles of vegetative and reproductive organs. Leaves generally function as long-lived photosynthetic tissues and may therefore support the prolonged synthesis and storage of volatile terpenes (PETROVIĆ et al., 2024). In contrast, floral tissues are primarily involved in reproduction, where volatile compounds contribute to ecological

Table 2 - Essential oil contents of *L. stoechas* from different altitudes (m), flowering period, and plant part (%).

Location	Altitude	Part	Period	EO (%)	SD	Groups
Yenicag	150	Leaf	BF	1.63	0.12	cd
			FF	1.80	0.17	bc
		Flower	BF	0.80	0.10	gh
			FF	1.03	0.06	fg
Alahan	300	Leaf	BF	1.60	0.17	cd
			FF	3.00	0.27	a
		Flower	BF	0.60	0.05	h
			FF	1.23	0.06	ef
Nergizlik	450	Leaf	BF	1.70	0.10	bcd
			FF	2.00	0.10	b
		Flower	BF	0.80	0.10	gh
			FF	1.40	0.05	de
Karlisu	600	Leaf	BF	1.20	0.05	ef
			FF	1.70	0.10	bcd
		Flower	BF	0.73	0.18	gh
			FF	1.43	0.05	de

BF: Before Flowering; FF: Full Flowering, SD=Standard deviation, Letters show the statistical groups.

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interactions such as pollinator attraction and plant–insect communication. Consequently, flowers may exhibit lower net EO accumulation on a dry-weight basis despite their important role in volatile emission.

As plants progressed from the pre-flowering (BF) to full-flowering (FF) stage, EO content increased consistently across all populations. The most pronounced increase occurred in Alahan, where leaf EO content nearly doubled from 1.60% to 3.00% (Table 2, Figure 1). This trend indicates that EO accumulation is closely associated with developmental stage and may reflect increased metabolic activity during reproductive development. Similar increases in EO production during flowering have been reported in several aromatic plant species and are generally attributed to enhanced terpene biosynthesis and the greater physiological demand for volatile compounds during anthesis (PRUSINOWSKA & SMIGIELSKI, 2014). The statistical grouping further supports this pattern, with flower samples collected before flowering consistently belonging to the lowest significance groups (g–h), whereas the highest floral EO contents were recorded at full flowering in Alahan (1.23%) and Karlisu (1.43%). Thus, full flowering represents the most favorable phenological stage for EO accumulation in *L. stoechas* under the environmental conditions examined.

The maximum leaf EO yield obtained in the present study (3.00%) is among the highest values reported for *L. stoechas*. It exceeds the maximum leaf yields reported from Italy (2.4%; ANGIONI et al., 2006), Moroccan wild populations (2.3%; AABOUCH et al., 2024), and introduced cultivars from Thailand (0.18–0.82%; INSAWANG et al., 2019). Such variation among studies likely reflects differences in genetic background, environmental conditions, plant material, and extraction methodology. Similarly, the EO yields observed in the present study were higher than those previously reported from Türkiye and Cyprus (TANKER et al., 2008; KÜÇÜK et al., 2019; ÖZTÜRK et al., 2005). Although, direct comparisons should be made cautiously because of differences in environmental conditions and experimental methodologies, the high EO yields recorded in the Hatay populations suggest that this region provides favorable conditions for EO production in wild *L. stoechas*. The

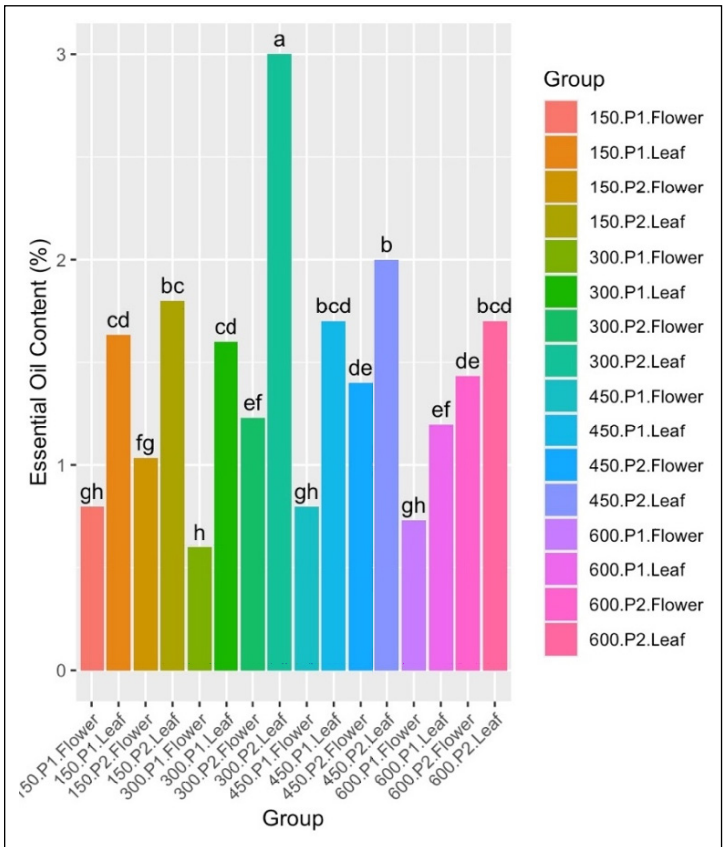


Figure 1 - Essential oil content variation and statistical groups.

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intermediate-altitude populations combined high EO yield with a relatively stable chemical profile, making them promising germplasm for breeding, conservation, domestication, and commercial cultivation.

Essential oil composition

The chemical profiles of the extracted essential oils showed considerable quantitative variation while remaining qualitatively similar across populations, plant organs, and phenological stages. The identified compounds accounted for 80.58–93.18% of the total essential oil composition (Table 3). Fenchone was the predominant constituent in all samples, ranging from 35.49% in Alahan leaves before flowering to 63.80% in Karlisu flowers at full flowering. The consistent predominance of fenchone, together with variation in accompanying monoterpenes, indicates that environmental and developmental factors affect the quantitative composition of the essential oil while maintaining a relatively stable overall chemotype.

Fenchone, camphor, and 1,8-cineole are major oxygenated monoterpenes derived from the plastidial methylerythritol phosphate (MEP) pathway and share common biosynthetic precursors (CZECHOWSKI et al., 2022). Their relative abundance may; therefore, reflect shifts in carbon partitioning among competing branches of monoterpene biosynthesis in response to environmental or developmental conditions. Although, the underlying biochemical mechanisms were not investigated, the observed compositional variation is consistent with the metabolic flexibility commonly reported for terpene biosynthesis in aromatic plants (LANIER et al., 2023).

The Alahan population exhibited a distinct camphor-rich profile, with camphor reaching 38.17% in leaves collected before flowering, making it the second most abundant constituent after fenchone. In contrast, Karlisu and Nergizlik contained considerably lower camphor proportions (5.68% and 3.78%, respectively) and relatively higher levels of 1,8-cineole. The highest 1,8-cineole concentration (22.23%) was recorded in Karlisu leaves at full flowering, followed by 19.78% in Nergizlik leaves. This contrasting abundance of camphor and 1,8-cineole may reflect differences in monoterpene biosynthesis associated with environmental conditions (ALI et al., 2017). As these compounds originate from a common precursor within the MEP pathway, their relative abundance may reflect shifts in metabolic flux among competing biosynthetic branches (WEBB et al., 2013). However, these biochemical mechanisms require further experimental validation. Myrtenyl acetate was also identified as a major constituent in Karlisu flowers before flowering, further supporting population-specific variation in essential oil composition.

Environmental variation across the altitudinal gradient may partly explain the differences in camphor and 1,8-cineole abundance. Temperature and solar radiation have been shown to influence monoterpene biosynthesis by modulating terpene synthase expression and activity (SANGWAN et al., 2001; LORETO & SCHNITZLER, 2010). Although these mechanisms were not directly investigated, they may contribute to the relatively high 1,8-cineole proportion in Karlisu and the greater camphor abundance in the lower-altitude Alahan population. Further physiological and molecular studies are needed to clarify these regulatory mechanisms.

A clear organ-specific and phenological pattern was also observed. Inflorescences collected at full flowering generally contained higher proportions of fenchone than the corresponding leaves, reaching 63.80% in Karlisu and 63.77% in Nergizlik. This pattern agrees with previous reports of organ-specific variation in *L. stoechas* essential oil composition (ANGIONI et al., 2006; DOMINGUES et al., 2023), including greater fenchone abundance in reproductive than vegetative tissues (SKOULA et al., 1996). The observed pattern may reflect developmental regulation of monoterpene biosynthesis during flowering, although the underlying mechanisms were not investigated. The chemotypic variation among the studied populations is also consistent with the diversity of *L. stoechas* essential oil profiles reported across Mediterranean and other regions. Fenchone/camphor and cineole/fenchone chemotypes have been described in populations from Korea (KIM & LEE, 2002), Greece (KOKKALOU, 1988), India (RAINA & NEGI, 2012), and Morocco (BENABDELKADER et al., 2011; ZRIRA & BENJILALI, 2003; BENALI et al., 2023). Collectively, these findings indicated that geographic origin, environmental conditions, plant organ, and developmental stage may contribute to the considerable chemical variability observed within the species.

To further explore multivariate relationships among the major essential oil constituents, Principal Component Analysis (PCA) was performed. PC1 was primarily associated with fenchone, fenchyl alcohol, and 1,8-cineole, whereas PC2 was more strongly associated with camphor and myrtenyl acetate. Together, PC1 and PC2 explained

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Table 3 - Variation in the essential oil composition of *L. stoechas* as affected by altitude, plant part, and phenological stage.

RT	Location	Yenicag				Alahan				Nergizlik			Karlisu				
	Plant Part	L	F	L	F	L	F	L	F	L	F	L	F	L	F	L	F
	Period	B.F.	B.F.	F.F.	F.F.	B.F.	B.F.	F.F.	F.F.	B.F.	B.F.	F.F.	F.F.	B.F.	B.F.	F.F.	F.F.
	Compounds																
3.92	α-pinene	0.53	0.61	0.56	0.59	0.76	0.80	0.64	0.37	0.96	0.94	0.62	0.63	0.83	1.10	0.87	0.65
4.53	ocimene	0.15	0.20	0.14	nd	0.11	0.15	0.13	nd	0.20	0.25	nd	0.20	nd	nd	nd	0.17
4.69	camphene	0.43	0.42	0.36	0.39	1.33	0.82	0.73	0.66	0.37	0.45	0.31	0.40	0.45	0.66	0.52	0.64
5.57	thujene	0.16	nd	nd	nd	0.21	0.15	nd	nd	0.18	0.48	0.23	nd	0.26	0.44	0.30	nd
7.95	limonene	0.75	0.69	0.67	0.68	0.73	1.04	0.81	0.72	0.98	1.53	0.94	0.99	0.92	1.25	1.16	0.69
8.15	1,8-Cineole	18.63	5.90	13.86	5.13	6.33	3.25	7.92	3.54	18.63	6.00	19.78	4.49	9.43	9.80	22.23	2.33
10.29	cymene	0.43	0.43	0.37	0.35	0.31	0.36	0.32	0.47	0.5	0.42	0.40	0.34	0.19	0.57	0.44	0.40
14.72	fenchone	51.33	64.05	56.66	61.26	35.49	44.73	39.95	50.92	54.80	59.95	47.94	63.77	56.95	50.24	50.19	63.8
16.46	linalool oxide	0.17	nd	0.21	nd	0.37	0.15	0.22	nd	nd	nd	0.27	0.23	nd	nd	0.22	nd
17.53	fenchyl acetate	0.23	0.96	0.32	0.73	0.19	0.44	nd	0.49	0.29	0.58	0.29	0.76	0.37	0.87	0.20	1.14
17.78	α-longipinene	0.26	0.19	0.4	nd	nd	nd	0.26	nd	0.32	0.20	0.38	0.18	nd	nd	0.26	0.20
18.25	α-cedrol	0.30	nd	0.39	nd	nd	nd	0.15	nd	0.43	0.17	0.68	0.18	nd	nd	0.32	0.30
19.19	camphor	10.12	5.96	8.69	8.02	38.17	27.43	32.82	14.27	3.78	7.55	5.94	6.80	7.93	5.64	5.68	7.03
20.68	linalool	0.41	0.23	0.32	0.28	0.61	0.76	0.58	0.61	0.22	0.81	1.16	0.62	0.65	0.77	0.82	0.31
21.71	bornyl acetate	0.47	0.68	0.31	0.53	2.61	1.55	1.07	0.89	0.20	0.57	0.24	0.69	0.67	1.50	0.47	0.29
21.92	fenchyl alcohol	0.76	1.95	0.86	1.80	0.30	0.58	0.41	0.44	0.52	1.26	0.59	1.50	0.61	1.15	0.51	1.80
25.44	verbenol	0.51	0.74	0.53	0.76	0.72	0.74	0.61	0.69	0.69	0.79	0.60	0.78	0.72	0.75	0.62	1.07
25.80	myrtenyl acetate	1.17	2.30	1.26	1.88	1.63	5.45	1.15	3.45	1.23	3.18	0.87	3.42	2.62	6.02	1.47	6.36
26.17	terpinenyl acetate	nd	0.42	0.27	0.36	nd	nd	nd	nd	nd	0.55	0.46	0.34	nd	3.16	0.60	nd
26.29	isoborneol	0.39	nd	0.15	0.31	0.48	0.38	nd	0.54	0.47	0.28	nd	nd	0.42	nd	nd	0.15
27.46	carvone	0.46	0.44	0.33	0.41	0.32	0.41	0.36	0.49	0.35	0.50	0.33	0.52	0.33	0.39	0.28	0.52
28.25	δ-Cadinene	0.37	0.24	0.63	0.43	0.24	0.23	0.29	nd	0.56	0.30	0.49	0.23	0.67	0.34	0.43	0.26
31.18	trans-carveol	0.21	0.35	0.27	0.39	0.26	0.31	0.27	0.42	0.30	0.39	0.29	0.37	0.23	0.45	0.28	0.40
35.81	caryophyllene oxide	0.44	1.00	0.42	0.49	0.16	0.35	0.47	nd	0.48	0.46	0.65	0.43	nd	nd	0.42	0.37
39.19	viridiflorol	2.70	1.27	2.94	2.00	1.85	2.04	2.44	1.61	2.74	2.24	3.34	2.22	2.66	1.90	2.57	0.39
Total		91.38	89.03	90.92	86.08	93.18	92.12	91.6	80.58	89.2	89.85	86.8	90.09	86.91	87.00	90.86	89.27

RT= Retention Time. Locations in terms of altitude Yenicag = 150 m; Alahan: 300 m; Nergizlik 450 m; Karlisu: 600 m. Plant Parts L: Leaf, F: Flower. Periods: B.F: Before Flowering; F.F: Full Flowering. Nd = not detected.

72.1% of the total variance (42.9% and 29.2%, respectively), providing a clear overview of chemical differentiation among populations (Figure 2).

The PCA revealed clear separation of Alahan from the other populations, with its position in the lower PC2 region and association with the camphor vector indicating a major contribution of camphor to its chemical differentiation. The relatively compact clustering of Alahan samples suggests comparatively limited within-population variation. Yenicag and Nergizlik showed partial overlap and were generally associated with fenchone, fenchyl alcohol, and 1,8-cineole, indicating chemical similarity despite differences in altitude. Karlisu occupied a distinct region of the PCA space, with some samples, particularly 13 and 15, positioned closer to the myrtenyl acetate vector. This distribution indicated population-specific variation in essential oil composition, although the contribution of local environmental conditions requires further investigation. Overall, PCA complemented the

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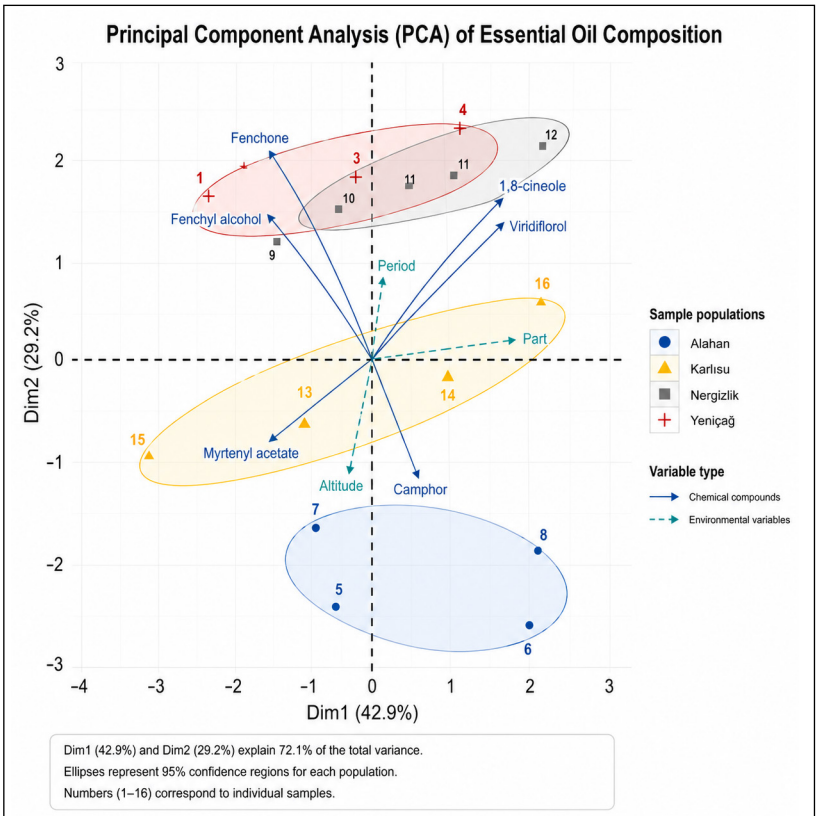


Figure 2 - Principal component analysis (PCA) biplot of essential oil composition in *L. stoechas* samples.

univariate analyses by demonstrating how the major constituents collectively contributed to population differentiation and supported the influence of altitude, plant organ, and phenological stage on *L. stoechas* essential oil composition.

CONCLUSION

In this study, *L. stoechas* subsp. *stoechas* samples collected from four altitudinal levels were evaluated across two phenological stages and two plant organs. Essential oil yield and composition exhibited clear variation among locations, developmental stages, and plant parts. Leaves generally showed higher essential oil yields than flowers, while fenchone and camphor were identified as the predominant constituents across all samples, with notable differences in their relative abundances among populations. Multivariate analysis revealed structured patterns of chemical differentiation among sampling sites and plant organs, highlighting associations between specific compounds and sample groupings. Overall, the findings demonstrated that ecological context, phenological stage, and plant organ are closely associated with essential oil variability in *L. stoechas* subsp. *stoechas*. These results provided a basis for future studies integrating anatomical, environmental, and biological analyses to further clarify the mechanisms underlying this variation.

DATA AVAILABILITY STATEMENT

The raw data supporting the findings of this study are available from the corresponding author upon reasonable request.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the conception and writing of the manuscript. All authors critically reviewed the manuscript and approved the final version.

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DECLARATION OF USE OF ARTIFICIAL INTELLIGENCE

Artificial intelligence was used to assist with language editing and improving the clarity and readability of the manuscript. All revisions were reviewed and verified by the authors to ensure that the scientific content remained unchanged.

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